

Claims:

1. A recombinant calf-Chymosin protein as set forth in SEQ ID No. 1.
2. A recombinant calf-Chymosin gene as set forth in SEQ ID No. 2.
3. The recombinant calf-chymosin gene as claimed in claim 2 encoding the protein comprising amino acid sequence of SEQ ID NO.1.
4. An *E.coli* comprising the recombinant chymosin gene of SEQ ID No. 2.
5. The *E. coli* as claimed in claim 4 is BL21 cell of *E.coli*.
6. An expression vector pET21b comprising recombinant calf-chymosin gene as set forth in SEQ ID No. 2.
7. A method for producing recombinant calf-chymosin protein as set forth in SEQ ID No. 1 which comprises steps of isolating calf-chymosin gene, cloning the same in bacterial expression vector pET21b, transforming said cloned vector into cells of *E.coli*, fermenting said *E.coli* to produce pro-chymosin, converting said pro-chymosin to chymosin and subsequently recovering the recombinant calf-chymosin.
8. The method as claimed in claim 7, wherein the calf-chymosin gene is obtained by isolating RNA from fourth stomach of calf tissue, synthesising a first strand of cDNA therefrom by treating the same with a reverse primer of SEQ ID NO.3 and then with a forward primer of SEQ ID NO.4.
9. The method as claimed in claim 8, wherein the cDNA is ligated at *sma*I site of pBSSK+ plasmid and then transformed into TOP10 cells of *E- coli*.
10. The method as claimed in claim 9, wherein said recombinant clones were identified and treated with a forward primer of SEQ ID NO.5 and reverse primer

of SEQ ID NO.6 containing Nde I and Hind III sites to obtain an amplified fragment.

11. The method as claimed in claim 10, wherein the amplified fragment is transformed into cells of *E.coli* for expressing chymosin gene.
12. The method as claimed in claim 11, wherein *E.coli* cells containing recombinant calf chymosin gene is fermented, the suspended cells produced on completion of fermentation are lysed, chilled and pH adjusted to about 8 before incubation at room temperature and the separation of supernatant containing prochymosin.
13. The method as claimed in claim 12, wherein the pH of supernatant is adjusted to about 2 for activation, further incubated for about 6 hrs and subjected to filtration to obtain filtrate.
14. The method as claimed in claim 13, wherein the filtrate is subjected to sodium chloride precipitation, then the precipitate is dissolved followed by the addition of sodium benzoate as preservative.